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The Rb Pathway and Cancer Therapeutics

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Abstract

The retinoblastoma gene, Rb, was originally identified as the tumor suppressor gene mutated in a rare childhood cancer called retinoblastoma (reviewed in [1]. Subsequent studies showed that Rb functions in a pathway that is often functionally inactivated in a large majority of human cancers. Interestingly, recent studies showed that in certain types of cancers, Rb function is actually required for cancer development. The intimate link between the Rb pathway and cancer development suggests that the status of Rb activity can potentially be used to develop targeted therapy. However, a prerequisite will be to understand the role of Rb and its interaction with other signaling pathways in cancer development. In this review, we will discuss the roles of Rb in proliferation, apoptosis and differentiation by reviewing the recent findings in both mammalian systems and different model organisms. In addition, we will discuss strategies that can be employed that specifically target cancer cells based on the status of the Rb pathway.

Rb AND E2F FAMILY PROTEINS

Mammalian Rb Family Proteins

In addition to Rb, the mammalian system has two other Rb related proteins, p107 and p130 (Fig. (**1**)). These three proteins are also referred to as the “pocket proteins” because their main sequence similarity resides in a domain, the pocket domain, which mediates interactions with viral oncoproteins as well as cellular proteins to exert the biological functions of this family. A non-conserved spacer region separates the conserved pocket domain into two parts. Interestingly, the spacer region of p107 and p130 but not Rb contain binding sites for cyclin/cdk complexes [2, 3].

Phosphorylation plays a key role in regulating the activities of the Rb protein. The Rb protein contains numerous phosphorylation sites that are phosphorylated by cyclinD/cdk4, cyclinE/cdk2, and cyclinA/cdk2 kinases during cell cycle progression [4-7]. Generally speaking, hypophosphorylated Rb is active in the inhibition of cell proliferation and tumor suppression while the hyperphosphorylated Rb is inactive. In addition to regulation by phosphorylation, the Rb family proteins have differing expression patterns depending on the stage of the cell cycle and the type of tissue. For example, p130 is most abundant in quiescent, differentiated cells and in early G1 [8]. p107 expression increases in mid to late G1 while Rb expression is prominent in both proliferating and non-proliferating cells [8].

The biological functions of Rb include tumor suppression, regulation of the cell cycle, differentiation, and apoptosis. These functions of Rb are mediated by its interaction with a large number of cellular proteins. Over 100 proteins have been reported to interact with the

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Rb protein [9], and most, if not all, of these interactions also involve the pocket domain. The best studied binding partners of Rb are the E2F transcription factors.

Mammalian E2F Transcription Factors

In the mammalian system there have been eight E2F transcription factors identified so far. E2F1 through 6 contain a DNA binding domain and dimerization domain and require DP proteins to form a heterodimer for DNA binding [10-12]. There are at least three types of E2F transcription factors. Activating E2Fs, E2F1, E2F2, and E2F3, are the most well known, and serve to promote progression into S phase when Rb is inactivated by actively promoting the transcription of cell cycle genes. Repressive E2F proteins, E2F4 and E2F5, act to repress the transcription of E2F target genes in a complex with the Rb family members. The third category of E2F proteins, E2F6, E2F7, and E2F8, can function to repress E2F target gene expression but they function independently of Rb binding. Interestingly, a recent study showed that E2F7 and E2F8 form homodimers and heterodimers with each other and function redundantly in repressing E2F1 expression during S and G2 phases [13].

Drosophila RB and E2F Proteins

There are two E2F (*dE2F1* and *dE2F2*), one DP (*dDP*), and two Rb family (*RBF* and *RBF2*) genes in the *Drosophila* genome [14-18]. The two *Drosophila* E2F proteins behave like the first two subgroups of the mammalian E2F proteins: dE2F1 mainly functions as a transcriptional activator [19, 20], comparable to the mammalian activating E2Fs proteins, while dE2F2 primarily mediates active repression, similar to the mammalian repressive E2F proteins E2F4 and 5 [20]. Furthermore, similar to the mammalian Rb protein that can bind to both the activating and the repressive E2F proteins, RBF can bind to both dE2F1 and dE2F2 proteins in *Drosophila* [20]. In contrast, RBF2 can only bind dE2F2 [16] analogous to the mammalian p107/p130 proteins that bind preferentially to the repressive E2F proteins (see Fig. (**2**)). Thus the Rb-E2F pathway is well conserved and is much simpler in *Drosophila* than in the mammalian systems.

Transcriptional Targets of E2f Proteins

Several microarray experiments have demonstrated that E2F transcription factors are involved in many cellular processes. In addition to regulating transcription of genes involved in promoting the G1-S transition, E2F proteins were found to be involved in the transcription of other cell cycle genes including those involved in DNA repair, mitosis as well as the spindle checkpoint. There were several other categories of genes that were regulated by E2F transcription including genes involved in apoptosis and the DNA damage checkpoint [21-23]. Another category of genes regulated by E2F are genes involved in the Ras pathway, which may indicate that E2F is involved in some developmental processes or that E2F regulates the cells response to mitogens or growth factors which are regulated by the Ras pathway [24, 25].

The relative simplicity of the *Drosophila* Rb and E2F proteins has made it an ideal system for the genome wide identification of Rb/E2F targets. Specifically, RNAi has been used to deplete the activating E2F (dE2F1), the inhibitory E2F (dE2F2), dDP, RBF or RBF2 from *Drosophila* cell lines [26] or specific tissues [27]. Although it is possible that secondary changes in gene expression could have occurred, the screen did detect actual changes in expression, suggesting that the E2F/Rb interactions were rate limiting under these conditions. A surprising result of this study was that very few genes showed both a decrease in expression in the absence of dE2F1 and an increase in expression in the absence of dE2F2, suggesting that most genes are more influenced either by repression or by activation rather than by both activities equally [26]. Most of the genes found to be regulated largely

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by dE2F1 were involved in processes similar to those found in the ChIP on CHIP screens, including the cell cycle, DNA replication and repair, mitosis, chromosome segregation, and checkpoints. However, the genes regulated primarily by dE2F2 were not involved in DNA repair or S-phase processes. While the functions of many of these genes are unknown, a surprising number appear to be unrelated to the cell cycle and instead are involved in development, including male and female specific genes. The importance of dE2F2 repression was confirmed by finding that expression of many of these genes was deregulated in *de2f2* null flies.

Further examination of repression of these differentiation genes has highlighted the complexity of Rb/E2F control of transcription and the importance of interactions with other factors. In comparing the requirement for dE2F2 and RBF2 to repress expression in *Drosophila* cell lines, ovaries, and embryos, microarray data showed that different genes were deregulated in each tissue with surprisingly little overlap, suggesting cell-type specific requirements for expression [27]. Comparison of dE2F2 and RBF2 binding to the promoters of these genes showed that in some cases the differences in expression correlated with dE2F2/RBF2 occupancy of the promoter, suggesting that cell type specific factors determine dE2F2/RBF2 distribution. In contrast, at other promoters dE2F2 and RBF2 were always present, suggesting that the functional relevance of promoter occupancy also varies with cell type. These findings emphasize the need to further understand the mechanisms determining Rb and E2F functions in specific contexts.

ROLE OF Rb FAMILY PROTEINS IN CELL PROLIFERATION

It is well established that Rb is a key inhibitor of entry into S-phase of the cell cycle, thereby regulating cell proliferation. The Rb family of proteins plays a role in regulating other stages of the cell cycle including G1 progression, S-phase entry and even exit from mitosis. The role of the Rb family of proteins can be both E2F dependent and independent. In addition, recent studies suggest that Rb also plays a role in transmitting the signals from both DNA damage and spindle checkpoint machinery to cell cycle machinery to prevent proliferation in the presence of damaged DNA. Interestingly, recent reports showed that Rb may be required for proliferation rather than acting as an inhibitor or proliferation in certain cell types [28]. Further understanding the different roles of Rb in cell proliferation, differentiation, and apoptosis could help us develop rational therapies either to prevent proliferation of cancer cells or to induce the apoptosis of cancer cells.

Regulation of G1/S

The Rb family proteins have differing levels of expression throughout G1 progression [29]. In early G1, p130 and p107 are expressed at high levels and are found in association with the repressive E2Fs. Due to being weak repressors and not having a nuclear localization signal, the repressive E2Fs require the Rb family proteins in order to repress gene expression [30]. In addition, Rb protein is also expressed in early G1 and associates with the activating E2F proteins. The Rb family proteins are hypophosphorylated in early G1 and associate with the E2F proteins to prevent expression of cell cycle progression genes.

The Rb family proteins recruit chromatin remodeling factors to regulate the expression of genes required for S-phase entry [31]. Some of the chromatin remodeling factors that are recruited are histone deacetylases (HDACs) and the SWI/SNF chromatin remodeling complex. As high levels of cyclin E drive the G1-S transition, cyclin E transcription is repressed in early G1. Rb protein is required for the recruitment of the HDACs in cyclin E transcriptional initiation sites [32]. Rb can also bind to components of chromatin remodeling complexes to affect gene expression. Rb was found in a complex with Brg1 and Brm which are ATP-dependent helicases that are involved in chromatin remodeling [33]. Rb can recruit

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Brg to specific promoters to inhibit E2F mediated transcription and to inhibit cell cycle progression. Interestingly, Rb recruits Brg to cyclin A but not cyclin E promoters. Since cyclin A drives S-phase progression and peaks in late S-phase, it is possible that this may be one mechanism by which the ordered expression of the cyclins is regulated [34]. Another way Rb may regulate E2F mediated transcription is via methylation of chromatin which can inhibit transcription. Rb was found in a complex with DNA methyltransferase 1 (DNMT1) and the repressive effects of Rb were enhanced by DNMT1 [35].

As cells progress to the middle of the G1 stage, cyclin D/cdk4 or cdk6 promote the hyperphosphorylation of the Rb family proteins resulting in dissociation of the Rb family proteins from E2F transcription factors allowing progression through G1 and eventually S-phase entry [6, 36]. In one current model the repressor E2Fs dissociate from the E2F target genes allowing gene expression and the activator E2Fs remain associated with the target genes and recruit histone acetyltransferases (HATs) to promote transcription of the cell cycle genes [37, 38]. In addition, activator E2Fs may occupy the promoter regions of all E2F target genes including those previously occupied by the repressor E2Fs.

Rb also has a role in regulating S-phase entry independent of transcriptional regulation via E2F proteins. Rb was found to associate with several proteins involved in the initiation of DNA replication including the DNA replication licensing factor MCM7, the replication factor RFC and the single stranded DNA binding protein, purα, suggesting that Rb plays a role in regulating the initiation of replication [39, 40]. CyclinD/Cdk4 was found to associate with MCM7 and this complex was catalytically active; however, CyclinD/Cdk4 did not phosphorylate MCM7. Instead it catalyzed the dissociation of Rb from the MCM7 complex in preparation for DNA replication [41].

Senescence is the non-reversible exit from the cell cycle. Rb seems to be particularly important in the regulation of senescence. Under conditions of stress p16 is upregulated leading to upregulation of Rb function. This activation leads to chromatin reorganization with repressive heterochromatin forming at loci containing E2F targets especially those regulating progression of the cell cycle, thus leading to exit from the cell cycle. The heterochromatin formation is not reversible, so p16 and Rb protein activation does not need to be retained [42]. Promoting cellular senescence could be one way to prevent proliferation of cancer cells.

Regulation of Mitosis and Cell Cycle Checkpoints

Rb also regulates the cell cycle beyond the G1-S transition. Work done in *Drosophila* showed that larvae mutant for the Rb homologe, Rbf, exhibited extensive defects in chromatin condensation in mitosis suggesting that Rb plays a role in mitosis. Rbf was found to interact with dCAP-D3 a component of the condensing II complex and was required for the accumulation of dCAP-D3 on chromatin [43]. These findings were found to be conserved in a human tumor cell line. Expression of Rb in an Rb deficient cell line promoted the association of hCAP-D3 with chromatin [43]. These findings provide one explanation for why inactivation of Rb will lead to genomic instability.

One recent study suggests that Rb plays a role in exiting mitosis and the initiation of G1 stage of the cell cycle. Rb may help regulate the targets of the anaphase promoting complex/cyclosome (APC/C) via a direct physical link between the Rb protein and the APC/C specificity factor, Cdh1. Cdh1 directs the destruction of the inhibitors of mitotic exit and G1 by ubiquitination via the APC/C and degradation via the proteosome. In this study Binne and colleagues found that Rb was required to direct the destruction of Skp2 the f-box protein of the Skp1cullin F-box protein, SCF, which is an E3 ubiquitin ligase. The targeted

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degradation of Skp2 led to the stabilization of p27(kip1), a CDK inhibitor, thereby promoting a G1 arrest [44].

Rb has also been shown to be involved in mediating a checkpoint induced response. One study showed that the spindle checkpoint kinase Mad2 is a direct transcriptional target of E2F1 and is overexpressed in Rb deficient cells. Overexpression of Mad2 can lead to chromosomal instability and tumorigenesis, contributing to cancer [45]. Rb may also play a role in the DNA damage checkpoint. Inoue and Taya showed that Rb was phosphorylated in an ATM, Chk1/2 dependent fashion in response to DNA damage on S612. Phosphorylation of S612 in response to DNA damage promotes the association of Rb with E2F and inhibits E2F mediated transcription, halting the cell cycle [46].

Although a role for Rb in inhibiting proliferation is well established, a recent study suggests that Rb is required for cell proliferation in certain cells. Work done by Morris and others showed that E2F1 can inhibit beta-catenin/T cell factor (TCF) dependent transcription of c-Myc and other targets that are important for cellular proliferation and survival. They also showed that E2F1 can upregulate the expression of proteins that result in beta-catenin degradation. Since the Wnt signaling pathway promotes cell proliferation and survival in some cell types, inhibition of E2F1 by Rb is required for the proliferation of these cells. E2F1 is also downregulated by CDK8, a colorectal oncogene [28]. This study supports the finding that Rb is often overexpressed or amplified in colorectal cancers [47, 48] unlike other cancer types in which Rb is often inactivated.

ROLE OF Rb FAMILY PROTEINS IN APOPTOSIS

In addition to regulation of the cell cycle, Rb also regulates other functions in cells and organisms including apoptosis. Rb may regulate apoptosis directly by controlling the expression of apoptosis regulators or indirectly by regulating cell cycle progression since cycling cells are more likely to undergo apoptosis than G1 arrested cells. Induction of apoptosis in cancer cells is one of the most promising areas of research in cancer therapy. Therefore identifying targets that would specifically induce apoptosis in Rb deficient cells could be a good strategy for the development of new cancer therapies.

Rb and Apoptosis in Mammalian Systems

Apoptosis may occur via a death receptor-dependent (extrinsic) or independent (intrinsic or mitochondrial) mechanism [49]. The mitochondrial pathway of cell death is mediated by the Bcl-2 family of proteins and caspases. The upstream activators of apoptosis are responsible for receiving and transmitting cell death signals, leading to the expression and/or activation of BH3 domain containing proteins. These proteins can signal to other Bcl-2 family proteins that can insert into the mitochondrial outer membrane and this leads to release of cytochrome-C (Cyt-C) [50]. Cyt-C binds to APAF-1 which leads to activation of the activating caspases including caspase 9 [51]. The activating caspases turn on the effector caspases to induce cell death. Apoptosis is further regulated by the inhibitors of apoptosis proteins (IAPs) which bind to and inhibit caspase function (reviewed in [52]. The inhibition of caspase activation by IAP proteins is counteracted by another protein released from mitochondria during apoptosis, Smac/Diablo [53, 54].

The Rb pathway can regulate apoptosis via transcriptional regulation of pro-apoptotic factors. E2F1 overexpression induces apoptosis via transcriptional activation of pro-apoptotic genes including Arf, p73, APAF-1, Smac/Diablo and Omi HTRA2 [55, 56]. Most BH3 only proteins are induced by E2F as well as some of the initiator and effector caspases [57]. E2F can also regulate apoptosis via stabilization of p53 protein by upregulating the levels of Arf and pin [56]. The regulation of proapoptotic target genes expression by Rb/E2F

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proteins can be further modulated by other regulators and signaling pathways. For example, GABP was found to bind directly to E2F1 and specifically inhibit E2F1 dependent apoptosis [58]. Another example is that DNA damage induced phosphorylation of E2F1 by the ATM/Chk2 pathway which results in activation of E2F1 transcriptional activity and promotes apoptosis [59-61]. In addition, DNA damage signals can lead to acetylation of Rb protein, which disrupt its binding to E2F1 and activates the pro-apoptotic transcriptional activity of E2F1 [62, 63].

Rb and Apoptosis in *Drosophila*

The general mechanisms of inducing apoptosis are conserved in *Drosophila* and mammalian systems. Activation of caspases induces cell death in both systems and both systems use an apoptosome complex. However, in *Drosophila* cyt-C does not seem to be necessary for apoptosis although it is still released from the mitochondria [64]. Reaper, Hid and Grim, which are inhibitors of the IAPs similar to Smac/Diablo, are the key regulators of apoptosis in *Drosophila* [65]. Reaper, Hid and Grim localize to the mitochondria and this is essential for their full cell death effect [66-68]. Apoptosis induction in *Drosophila* also involves disruption of the mitochondria similar to the mammalian system [64].

The role of the Rb (Rbf) pathway in apoptosis is also conserved in *Drosophila*. Overexpression of dE2F1 is pro-apoptotic in most developing cells and results in transcription of dArk/Apaf1, and Reaper [69, 70]. Rbf is able to suppress apoptosis in an E2F dependent manner by limiting the expression of Hid [71, 72]. The effects of the Rb pathway on apoptosis are dependent on the developmental context of the cell. For example, in the larval eye disc, removal of Rbf results in increased apoptosis but only in cells along the morphogenetic furrow [19]. The conservation of the apoptotic pathways along with the ease of carrying out genetics screens makes *Drosophila* a good system for identifying potential new drug targets that would result in the induction of apoptosis in the absence of Rbf.

Moon and colleagues showed that in the absence of Rbf function the E2F dependent apoptosis was dependent on *hid* and *reaper* [73]. In support of this finding, Hid was identified in a screen to identify novel genes that regulated apoptosis in Rbf deficient cells [72]. In addition, our lab showed that *hid* was deregulated in *rbf* mutant larvae and that *hid* was repressed directly by Rbf/E2F proteins via association with an E2F binding site in the *hid* enhancer [72]. In one screen for suppressors of dE2F1 mediated apoptosis, the Dyson lab found AAC11 (mammalian homologue Api5) AAC11 drives cell death in an E2F dependent fashion but does not affect dE2F1 mediated transcription of cell cycle or pro-apoptotic genes suggesting that dE2F1 mediated apoptosis may be regulated at least to some extent in a non-transcriptional fashion [74]. In support of this finding, Bantam, a miRNA that regulates the translation of Hid, can modulate the survival of *rbf* mutant cells [72]. These results suggest that there are potential molecular drug targets that can allow us to induce the specific apoptosis of Rb mutant cells.

ROLE OF Rb FAMILY PROTEINS IN DIFFERENTIATION

Results from model organisms have shown that the Rb pathway is also involved in regulating the differentiation of cells in developing organisms. In developing cancer therapies, one way to stop tumor growth could be to reactivate differentiation pathways so that the tumor cells undergo terminal differentiation and stop proliferating. Understanding the role of Rb pathway in regulating differentiation will potentially give us novel targets for promoting terminal differentiation and blocking proliferation in cancer cells.

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Mammalian

There is some evidence that the Rb pathway regulates differentiation in mammalian cells. The Majority of the evidence of Rb in differentiation may be related to its role in regulating cell cycle exit. Cells lacking Rb function cannot exit the cell cycle and may continue to proliferate when they should be terminally differentiated. For example, Rb null hematopoietic cells cannot fully differentiate and this may result in myloproliferative disorders which could lead to an increased number of precursor cells and more tumors [75]. Rb may also coordinate cell cycle exit with differentiation. In Rb null skin cells, the cells continue to divide despite having markers for being differentiated [76], and in the sensory hair cells of the ear, removal of Rb results in fully differentiated cells continuing to proliferate [77]. Work done by Guo and colleagues showed that Rb is required for the quiescence and differentiation of enterocytes in the intestine. Deletion of Rb protein in the small intestine enterocytes resulted in ectopic cell cycle reentry and while these cells had a higher rate of apoptosis, the remainder of the cycling cells did not completely differentiate which may lead to the continued proliferation of the undifferentiated cells [78]. Therefore, inactivation of Rb in the small intestine could lead to uncontrolled cell growth of undifferentiated cells. In conditional knockout mice where Rb deficient mice could live until birth there was abnormal development and impaired ossification of bones. The Rb deficient osteoblasts impaired cell cycle exit suggesting that Rb was required for linking cell differentiation and cell cycle exit [79]. These results suggest that Rb has an important role in maintaining or establishing cell cycle exit and the quiescent state of differentiated cells.

There is also evidence that Rb may play a more direct role in differentiation. In MEFs that have been activated to become adipocytes, removal of Rb blocks while removal of E2F4 promotes differentiation. Interestingly, E2F4 loss does not override the differentiation defect resulting from Rb loss even though it completely suppresses the proliferation defect, suggesting that Rb promotes adipogenesis independent of its cell cycle role [80]. Additionally, mutation of Rb in mouse lungs led to more neuroendocrine cell differentiation, which suggested that Rb can regulate differentiation in lung cells by specifically inhibiting neuroendocrine cell fate [81]. Rb has been shown to have protein-protein interactions with proteins other than the E2F family of proteins and many of these interactions are with proteins involved in differentiation. Rb can interact with the transcription factor involved in differentiation of muscle tissue, MyoD [82]. Rb may help promote differentiation of muscle tissue by coordinating with MyoD to induce transcription of muscle target genes. There is some evidence that shows that acetylation of Rb may help promote its role in muscle development, but not affect the role of Rb in cell cycle. The acetylation mutant is able to arrest the cell cycle, but is not able to cooperate with MyoD to regulate MyoD transcription [83]. In rat neural stem cells overexpression of Rb or p130 affected the lineage specification of differentiating cells, but did not inhibit cell proliferation or apoptosis [84]. These results suggest that the Rb family proteins can regulate differentiation independent of their role in the cell cycle. Consistent with this, cell cycle independent roles of Rb proteins have also been observed in *C elegans* and in *Drosophila* systems, suggesting that the role of Rb proteins in differentiation could also be conserved.

C. elegans

The *C. elegans* system is an excellent model system for studying the role of specific genes in a developmental context. *C. elegans* contains the homologues of the Rb pathway including Lin-35 (Rb), EFL-1 (E2F transcription factor), and DPL-1 (DP). Mutation of these genes has revealed a role for the Rb pathway in embryonic development and oocyte maturation, and Rb-E2F mediated transcription repression regulates the expression of developmental genes to the proper context. The Rb pathway was found to play a role in *C. elegans* vulval development. In *C. elegans*, epidermal growth factor (Lin-3) signaling is essential for

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appropriate vulval development. Lin-3 signals via RAS and MAPK signaling pathways to activate the transcription factor Lin-1 in the 22 cells that will form the vulva. Null mutants of Lin-1 result in multipule vulval type structures or Muv (reviewed in [85]. In a screen to find other components of this pathway that resulted in Muv phenotype, the Horvitz lab identified two classes of mutants, class A and B. Interestingly, one mutation in class A in combination with one mutation in class B resulted in a synergistic Muv phenotype, but multiple mutations in the same classes resulted in a wild type phenotype [86]. *Lin-35*, the Rb homologue in *C. elegans*, was identified as a class B gene suggesting that the Rb pathway plays an important role in regulating differentiation. The identification of the Rb mutation in one of the two classes of the synergistic Muv mutants indicates that the role of Rb in vulval development is at least partially redundant with other mechanisms.

Drosophila

Drosophila is a good model organism for studying the role of the Rb pathway in development. Similar to the role of Rb in differentiation in the mouse, deletion of *rbf* was shown to have mild differentiation defects on its own [19, 87]. This suggests that the role of Rbf in differentiation in flies may also involve partially redundant mechanisms as observed in *C. elegans*. Using a mosaic eye screen, our lab was able to identify genes that are required for the proper differentiation of *rbf* mutant cells. In particular, a gene called *rhinoceros* (*rno*) was identified to have a role in regulating eye cell differentiation in the absence of Rb. Tissue mutant for both *rbf* and *rno* displayed developmental defects in the larval, pupal and adult stages of development. Notch signaling is critical for the proper R8 determination. It was found that *rbf* and *rno* exhibited partially redundant regulation of the expression of Dl, the ligand of Notch signaling. In addition, this regulation was dependent on the dE2F1 transcription factor (Steele *et al* , submitted). Using this type of screening, it is possible that novel roles for Rb in regulating differentiation can be uncovered, and proteins that work in tandem with Rb to promote differentiation can be identified.

THE STATUS OF THE Rb PATHWAY AS A GUIDE FOR CANCER THERAPIES

The general approaches employed in cancer therapies are to induce apoptosis of cancer cells or to inhibit their cell proliferation/induce differentiation. Successful cancer therapeutic drugs such as Gleevec that have relatively low side effects generally have the abilities to preferentially target the cancer cells while mostly sparing normal cells. The development of such successful cancer drugs require that distinct features of the cancer cells be targeted. As shown in Fig. (**3**), there are at least three different functional states of Rb in cancer cells. While a majority of cancers have an inactivated Rb pathway, either by mutation/deletion of Rb or by its functional inactivation (Fig. (**3**), category I and II), a small subset of cancers require functional Rb and may have overexpression or amplification of Rb (Fig. (**3**), category III). Depending on the status of the Rb pathway, different strategies can potentially be used to specifically target cancer cells (see Fig. (**3**)).

Inducing apoptosis is a commonly used strategy in cancer therapies. Since the inactivated Rb pathway is a common feature that distinguishes cancer cells from normal cells for a majority of cancers, an approach that specifically kills Rb deficient cells can potentially be useful to treat large majority of cancers. Because the expression of a number of apoptosis regulators are controlled by E2F, Rb deficient cells are often somewhat sensitized to apoptosis and are more dependent on survival signals. However, currently we do not know how to specifically kill cancer cells with an inactivated Rb pathway and a lot of research will be required to identify these types of drug targets. It is likely that studies using model organisms to identify genes that regulate the apoptosis of Rb mutant cells could lead to such

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new drug targets. For example, identification of *hid* as a modifier of *rbf* null cells in *Drosophila* uncovered the role of the *bantam* miRNA in regulating the apoptosis of *rbf* cells. While removal of *bantam* alone was not sufficient to induce apoptosis, increased apoptosis was observed in *bantam, rbf* double mutant cells [72]. Although these studies were carried out in *Drosophila*, it is likely that conserved regulators of apoptosis of Rb deficient cells can be identified in such studies, which will potentially allow specific induction of apoptosis to cells that have deficient Rb pathway without significant effect to cells with normal Rb pathway activity.

Although a majority of human cancers have an inactivated Rb pathway, functional Rb is actually required in a subset of cancers to maintain the proliferation and prevent apoptosis of cancer cells (Fig. (**3**), category III). For such kind of cancers, a possible approach will be to develop small molecule inhibitors of Rb function. Temporary inhibition of the Rb function can potentially lead to the killing of cancer cells or sensitization of th ese cancer cells to other chemotherapeutic agents. However, the success of such an approach hinges on the possibility that these cancer cells will be more sensitive to the loss of Rb function than their normal cell counterparts in the body. The observation that Rb is often overexpressed or amplified in colorectal cancers [47, 48], a cancer type that requires Rb function, certainly suggest the possibility that colon cancer cells could be more sensitive than wild type cells to the loss of Rb function. Further research will be needed to determine the feasibility of this approach.

While most research on drug therapy for cancer focuses on inducing apoptosis, other viable options include inhibiting cell proliferation, inducing cellular senescence, or reactivation of differentiation pathways so that the cells undergo terminal differentiation and stop proliferation. For cancers in which Rb is not irreversibly inactivated, it may be possible to reactivate Rb function to halt tumorigenesis. Much more research needs to be done to fully understand the cell types and cellular context in which this would work. Understanding the role of the Rb pathway in regulating differentiation will potentially give us novel targets for promoting terminal differentiation and blocking proliferation in cancer cells.

In summary, it is possible that the Rb status in cancers can be used to develop new cancer therapeutic drugs that could lead to better treatments with potentially fewer side effects. However, much more work will be needed to understand the control of cell proliferation, apoptosis and differentiation in the presence or absence of Rb.

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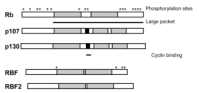

Fig. (1). The mammalian and *Drosophila* Rb family proteins

The Rb family of proteins in mammals consists of Rb, p107 and p130, and in *Drosophila* contains RBF and RBF2. The pocket domain, which is shaded in grey, is conserved and is responsible for most protein-protein interactions. A cyclin/cdk interaction motif (shaded in black) is conserved in the spacer region of p107 and p130 but absent in Rb protein. The activity of Rb proteins is controlled by phosphorylation at numerous phosphorylation sites (indicated for Rb and RBF by *). The phosphorylation sites in other Rb family members have not been precisely mapped.

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Fig. (2). Similarities between the Rb and E2F proteins in mammals and *Drosophila*

In mammals the E2F family is composed of eight E2F proteins, E2F1 through 8 and three DP proteins, DP1, DP2, and DP4. In *Drosophila* it consists of two E2Fs, dE2F1, dE2F2, and one DP protein, dDP. The eight mammalian E2F proteins can be divided into three groups: activating E2Fs (E2F1, E2F2, and E2F3), repressive E2Fs (E2F4 and E2F5), and Rb independent E2Fs (E2F6, E2F7, and E2F8). The mammalian activating E2Fs interact only with Rb. Similarly, the *Drosophila* activating E2F, dE2F1, interacts only with RBF. The mammalian repressive E2Fs interact with p107 and p130. In addition, E2F4 can also interact with Rb protein. Similarly, the *Drosophila* repressive E2F, dE2F2, interacts with both RBF and RBF2.

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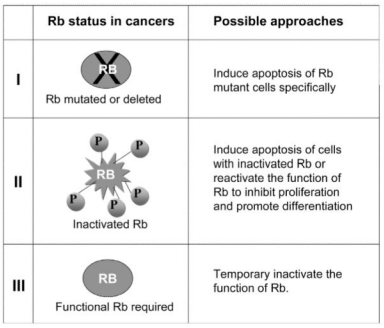

Fig. (3). The Rb pathway status in cancers and possible therapeutic approaches

There are at least three different functional states of Rb in cancer cells. While a majority of cancers have an inactivated Rb pathway, either by mutation/deletion of Rb or by its functional inactivation (category I and II), a small subset of cancers require functional Rb (category III). Depending on the status of the Rb pathway, different strategies can be potentially used to specifically target cancer cells. See text for more detailed discussion.

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